A Validated High Performance Liquid Chromatography Method for the Simultaneous Analysis of Guaifenesin, Ambroxol and Loratidine in Bulk and Liquid Dosage form .

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2018-2019 1439-1440

ABSTRACT

-A simple and sensitive HPLC method for simultaneous quantification of guaifenesin, ambroxol and loratidine in bulk and liquid dosage form was developed and fully validated.(1)

- The separation and quantification was performed using a Kromasil C8 (250 × 4.6 mm, particles 5 µm) HPLC column

- The detector was set to a wavelength of 290 nm and the column oven was maintained at 30 °C.(2)

ABSTRACT

* - The calibration plots were linear over the concentration ranges from 50-150 µg/mL, 30-90 µg/mL and 5-15 µg/mL for guaifenesin, ambroxol and loratidine, respectively.
* - Developed method was successfully applied for the quantification of the above three drugs in liquid dosage form.
* - The excipient did not interfere with drug peak

• INTRODUCTION

Guaifenesin *, glyceryl ether of guaiacol.*

- Is an expectorant used to lessen chest congestion caused by the common cold, infections, or allergies.(4)

- ***Guaifenesin*** *clears chest congestion by loosening and reducing the viscosity of phlegm, increasing the volume of phlegm and making coughs more productive.(4)*

- Chemically Guaifenesin is known as 3-(2-Methoxyphenoxy)-1,2-propanediol.

- Analytical methods for the determination of guaifenesin, either alone or in combination with other drugs, include spectrophotometry , gas chromatography, capillary electrophoresis-mass spectrometry , X-ray diffraction , voltammetry and HPLC .(5)

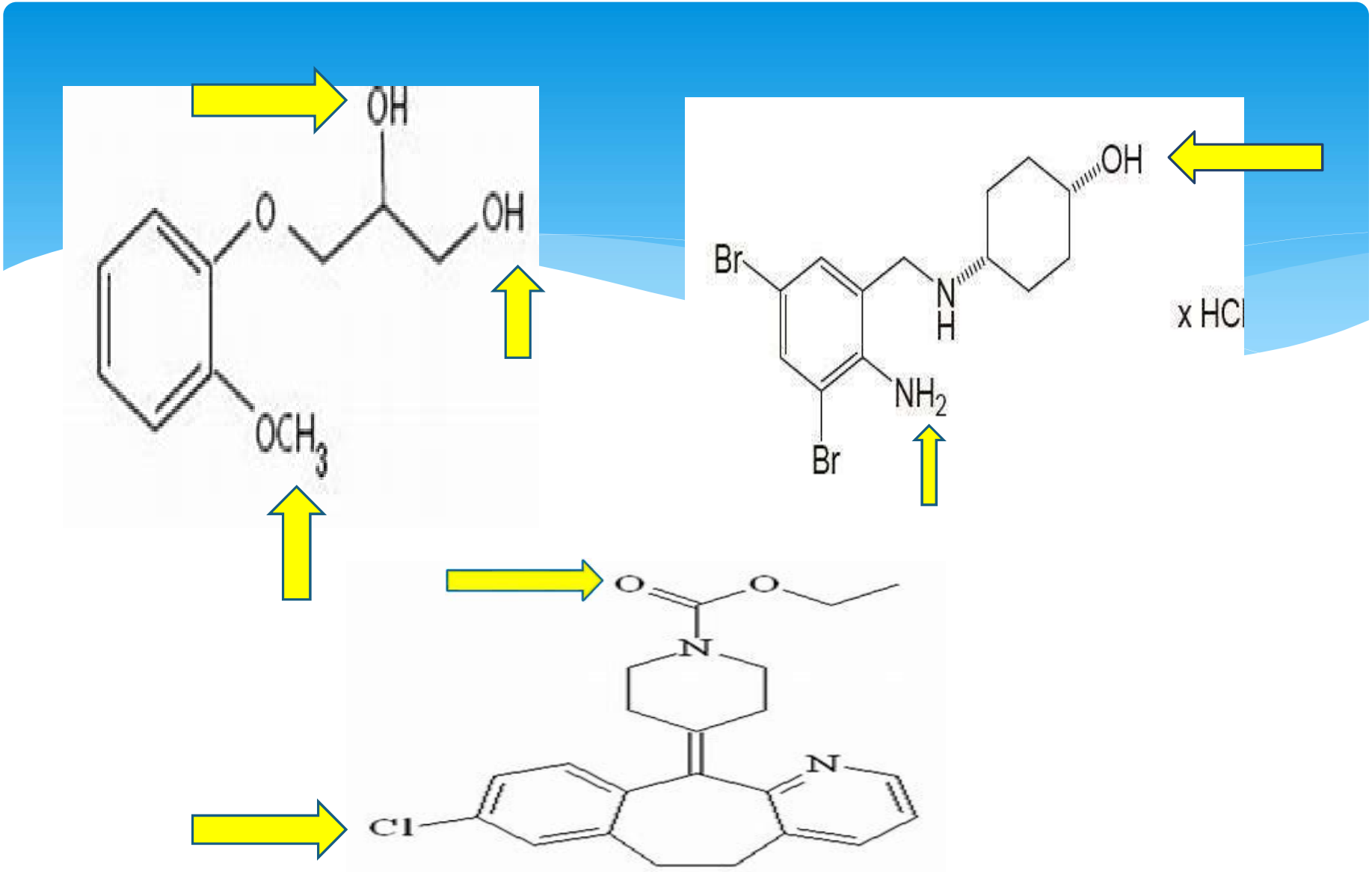
- ***Ambroxol hydrochloride***, *chemically known as trans-4 (2-amino-3, 5-dibromobenzyl -amino-cyclohexanol hydrochloride.*

-Is a potent mucolytic and mucokinetic , capable of inducing bronchial secretion

Loratadine *chemically known as ethyl 4-(8-chloro-5,6-dihydro 11H-benzo(5,6) cyclohepta (1,2-b) pyridin-11-ylidene)-1- piperidine carboxylate.*

-Is a second-generation piperidine histamine H1receptor antagonist with anti-allergic properties.

- It is used in the treatment of allergic rhinitis, urticaria and reduce the symptoms of hay fever for the short term.(8)



Chemical structure of A) Guaifenesin B) Ambroxol C) Loratidine

Chromatographic conditions

Kromasil C8 (250 × 4.6 mm; 5 µm particle size) analytical column was used for separation and analysis of guaifenesin, ambroxol and loratidine.(13)

- The column temperature was maintained at 30 ± 1oC.

- Mobile phase consisted of 0.1% orthophosphoric acid and acetonitrile in the ratio of (60:40 v/v, respectively).

- The separation was done under isocratic elution with flow rate maintained at 1.2 mL/min.

-The injection volume was 10 µL.

-The guaifenesin, ambroxol and loratidine were analyzed using a PDA detector set at 290 nm

• Sample solution

Commercial syrup (contain 50 mg guaifenesin, 30 mg ambroxol and 5 mg loratidine per 5 mL of syrup) was purchased from local pharmacy store.

- The syrup was shaken thoroughly to make homogenous mixture.

-A volume of the syrup equivalent to 50 mg guaifenesin, 30 mg ambroxol and 5 mg loratidine was transferred accurately into a 100 mL volumetric flask containing 30 mL of mobile phase.(14)

-The contents of the flask was shaken for about 10 min and diluted to volume with the mobile phase.

-The solution was then filtered through a 0.45 µm millipore filter.

-The above solution was appropriately diluted with the mobile phase to get a final concentration of 100, 60 and 10 µg/mL of guaifenesin, ambroxol and loratidine, respectively.(15)

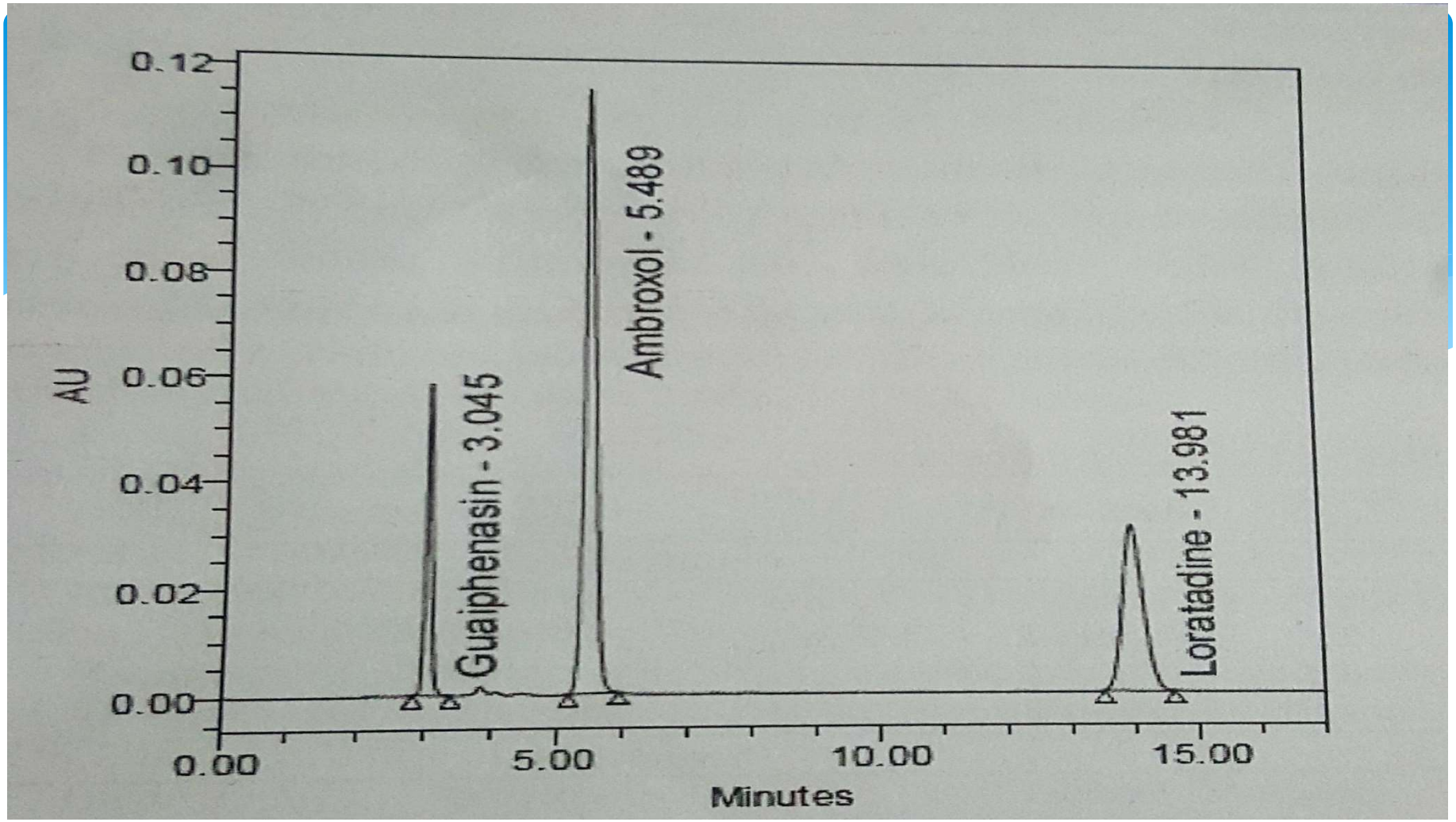
• RESULTS AND DISCUSSION

-For optimization of the chromatographic conditions and to obtain symmetrical peaks with better resolution and with acceptable system suitability results, various chromatographic conditions such as composition of mobile phase, flow rate and two different analytical columns were applied to guaifenesin, ambroxol and loratidine combination.(17)

-Among the tested analytical columns [ACE C8 (150 mm x 4.6mm, 5 µm particle size) and Kromasil C8 (250 × 4.6 mm; 5 µm particle size)] during preliminary investigations, Kromasil C8 (250 × 4.6 mm; 5 µm particle size) was the most appropriate column for simultaneous analysis of guaifenesin, ambroxol and loratidine.(18)

-In the preliminary trials different compositions of mobile phases consisting of 0.1 M dipotassium hydrogen phosphate/acetonitrile and 0.1% orthophosphoric acid/acetonitrile, different ratios and different flow rates of these solutions were employed to achieve the best system suitability results.(20)

- *Finally, the mobile phase composition of 0.1% orthophosphoric acid: acetonitrile in the ratio of 60:40 v/v with a flow rate of 1.2 mL/min was shown to have good resolution with minimal tailing factor in acceptable range.(23)*
- *The column temperature of 30 oC and detector wavelength set at 290 nm was chosen as suitable condition.(24)*
- *Under the mentioned chromatographic conditions highly symmetrical and sharp peaks of guaifenesin, ambroxol and loratidine were obtained at retention times of 3.045 min, 5.489 min and 13.981 min, respectively.(25*



Chromatogram of guaifenesin, ambroxol and loratidine combination standard solution under optimized chromatographic condition(30)

• System suitability

- System suitability test was performed from five replicate injections of a standard solution (40)
containing 100, 60 and 10 µg/mL of guaifenesin, ambroxol and loratidine, respectively.
- All peaks were well resolved. The precision of injections for all peaks were acceptable.(45)
- The percent relative standard deviations of the peaks area responses were measured.
- The USP tailing factor, USP resolution and USP plate count were also calculated.(46)
- The results of system suitability in association with the required limits are presented in Table.

.Table: System suitability(48)

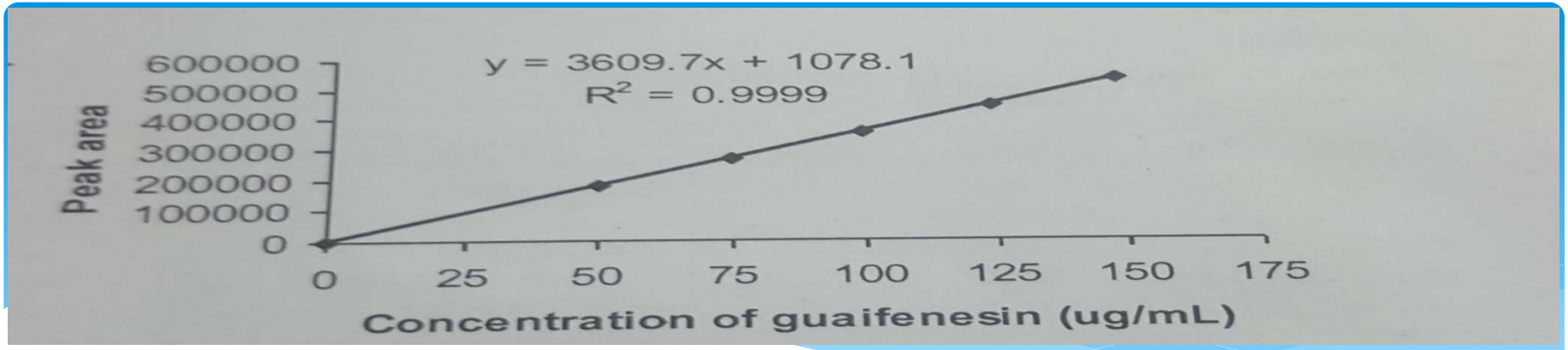
Parameters	Results			Recommended limits
	Guaifenesin	Ambroxol	Loratidine	
Retention time	3.045	5.489	13.981	-
Peak area	360940 (%RSD – 0.8)	1363778 (%RSD – 0.4)	710371 (%RSD – 0.3)	RSD ≤1
USP resolution	25.03	10.38	18.30	> 1.5
USP plate count	5517	5236	8467	> 2000
USP tailing factor	1.22	1.06	1.10	≤ 2

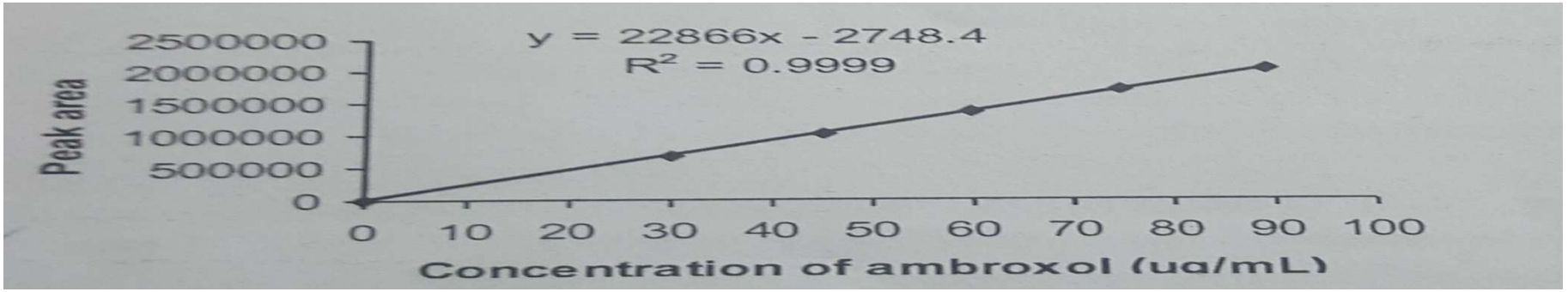
Linearity

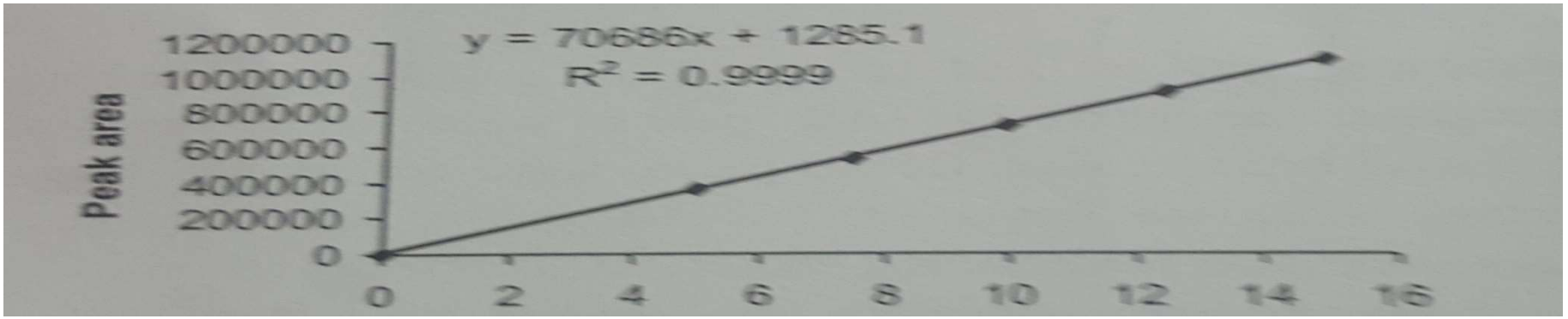
-The linearity test was performed using five different amounts of guaifenesin, ambroxol and loratidine in the range 50-150 µg/mL, 30-90 µg/mL and 5-15 µg/mL, respectively.(55)

-Solutions corresponding to each concentration level were injected in duplicate and linear regression analysis of the guaifenesin, ambroxol and loratidine peak area vs guaifenesin, ambroxol and loratidine concentration were calculated.(56)

- The results are summarized in Figures below


y = 3609.7x + 1078.1
R² = 0.9999
Peak area
600000
500000
400000
300000
200000
100000
0
0
25
50
75
100
125
150
175
Concentration of guaifenesin (ug/mL)


y = 22866x - 2748.4
R² = 0.9999
Peak area
2500000
2000000
1500000
1000000
500000
0
0
10
20
30
40
50
60
70
80
90
100
Concentration of ambroxol (ug/mL)


y = 70686x + 1285.1
R² = 0.9999
Peak area
1200000
1000000
800000
600000
400000
200000
0
0
2
4
6
8
10
12
14
16

Sensitivity

-The sensitivity of the developed method was assessed by determining Limit of quantification (LOQ) and detection (LOD).(63)

-The LOQ and LOD were predicted by the following formulae

(a) LOQ = 10 σ / S

(b) LOD = 3.3 σ / S

Where σ = standard deviation of response

S = slope of the calibration curv

-The LOD was found to be 0.754 µg/mL, 0.231 µg/mL and 0.145 µg/mL

whereas LOQ was found to be 2.513 µg/mL, 0.769 µg/mL and 0.483 µg/mL for guaifenesin, ambroxol and loratidine, respectively.

-The results reveal satisfactory sensitivity of the developed method.(65)

Precision

-The precision of the developed method was demonstrated by intra-day variation studies.(68)

- For this purpose, six repeated injections of standard solutions (guaifenesin-100 µg/mL; ambroxol-60 µg/mL; loratidine-10 µg/mL) were made.(69)

- The response of guaifenesin, ambroxol, loratidine and their percentage relative standard deviation (%RSD) were calculated. From the results, the developed method was considered to be precise.(70)

Guaifenesin		Ambroxol		Loratidine	
Peak area	%RSD	Peak area	%RSD	Peak area	%RSD
364354		1369971		712382	
363644		1377191		714875	
361943	0.29	1359782	0.41	711901	0.16
362672		1371184		713348	
363559		1368971		713069	
361704		1367314		711824	

Accuracy

-The accuracy of the method was determined via recovery experiments.(71)
- The accuracy of the proposed method was demonstrated by preparing samples spiked with 50%, 100%, and 150% of the test concentration of guaifenesin, ambroxol and loratidine.(72)

- Each concentration level was analyzed thrice. Mean percent recovery and percent RSD were calculated for each concentration.(73)

-Recovery of individual components was well within the acceptable limit .(74)
-From the data obtained, added recoveries of drugs were found to be accurate.(75

Accuracy level	µg/mL added	Peak area	µg/mL found	% Recovery	% Mean
			Guaifenesin		
50%	50.00	181685	50.03	100	100
	50.00	180916	49.82	100	
	50.00	181188	49.90	100	
100%	100.00	363957	100.23	100	100
	100.00	361611	99.58	100	
	100.00	364536	100.39	100	
150%	150.00	543535	149.69	100	100
	150.00	545074	150.11	100	
	150.00	544511	149.95	100	
			Ambroxol		
50%	30.00	683406	29.92	100	100
	30.00	682872	29.89	100	
	30.00	682683	29.88	100	
100%	60.00	1373533	60.13	100	100
	60.00	1369581	59.95	100	
	60.00	1358886	59.49	99	
150%	90.00	2057259	90.06	100	100
	90.00	2047357	89.62	100	
	90.000	2061957	90.26	100	
			Loratidine		
50%	5.00	354211	4.98	100	100
	5.00	356071	5.00	100	
	5.00	355322	4.99	100	
100%	10.00	714041	10.03	100	100
	10.00	712498	10.01	100	
	10.00	713146	10.02	100	
150%	15.00	1072341	15.07	100	100
	15.00	1068522	15.01	100	
	15.00	1067093	14.99	100	

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CONCLUSION

The optimal chromatographic conditions for separation and simultaneous quantification of guaifenesin, ambroxol and loratidine were achieved on an Kromasil C8 (250 × 4.6 mm; 5 µm particle size)(82)
analytical column with a isocratic elution at a flow rate of 1.2 mL/min, using 0.1% orthophosphoric acid:acetonitrile (60:40 v/v) as mobile phase and detection set to a wavelength of 290 nm.

-The method was simple and does not require preparation of buffer.
-The proposed method has the advantages of being sensitive, high resolution factor and less tailing factor than the reported HPLC methods.(83)
- All measured parameters of the validation reveal the suitability of developed HPLC method for the simultaneous analysis of guaifenesin, ambroxol and loratidine in bulk and liquid pharmaceutical preparation.(84)

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Thank you